Discover the Microbes Within! The *Wolbachia* Project: Citizen Science and Student-Based Discoveries for 15 Years and Counting

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The Elizabeth W. Jones Award for Excellence in Education recognizes an individual who has had a significant impact on genetics education at any education level. Seth R. Bordenstein, Ph.D., Centennial Professor of Biological Sciences at Vanderbilt University and Founding Director of the Vanderbilt Microbiome Initiative, is the 2020 recipient in recognition of his cofounding, developing, and expanding Discover the Microbes Within! The *Wolbachia* Project.

KEYWORDS citizen science; DNA; hands-on research; problem-based learning; STEM workforce

THE *Wolbachia* Project is a unique science experience that brings real-world, genetic research to middle schools, high schools, colleges, and citizen scientists. Participants engage in inquiry, discovery, and a culture of excellence by joining *Wolbachia* researchers to study and understand the prevalence of *Wolbachia*-infected arthropods while conducting biotechnology techniques such as polymerase chain reaction, gel electrophoresis, Sanger sequencing, bioinformatics, and evolutionary analysis – all common at the college and professional levels. In this new era of increasingly digital, remote, and asynchronous learning, The *Wolbachia* Project launched online resources and technical support to continue scientific literacy and problem-based learning. The *Wolbachia* Project welcomes all teachers, students, citizen scientists, and mentors to become *Wolbachia* Project Scientists. Free primers, controls, loaner equipment, and support are provided. To get involved, see the lab series, sign up for news, donate support, and learn more about the project, visit https://vu.edu/wolbachia.

The United States recognizes the importance of Science, Technology, Engineering, and Mathematics (STEM) education in its K-12 classrooms, leading students to successful, in-demand jobs as part of the growing American STEM workforce. This importance is highlighted and reinforced by reports calling for the high-priority need for student engagement and ownership of scientific research, namely by advancing accessible learning activities that invite intentional play and risk, interdisciplinary approaches to solving grand challenges, and flexible and inclusive learning spaces (Tanenbaum 2016). In addition, there are steadily increasing efforts at the state and school district level to implement project-based learning, integrate cross-cutting concepts into curricula, enable hands-on learning, and forge partnerships with scientists and industry leaders in the community (NGSS Lead States 2013). However, during this time when districts and classrooms are transforming STEM education for their learners, the coronavirus disease (COVID-19) pandemic brought science out of the physical classroom like no other time in modern history. Increasing numbers of classrooms adopted remote learning models and faced new challenges for hands-on, project-based, science education. It is more important than ever for education systems to find solutions that utilize and adapt best practices for promoting STEM retention, student engagement, hands-on discovery, and investment in science and scientific experiences.

Discover the Microbes Within! The *Wolbachia* Project is a proven, integrated lab series designed to bring real-world, scientific research into classrooms and engage participants in the biodiversity of their natural and built environments, critical thinking, the scientific method, biotechnology, animal–microbe–virus symbioses, and genetics. It was established

doi: https://doi.org/10.1534/genetics.120.303649
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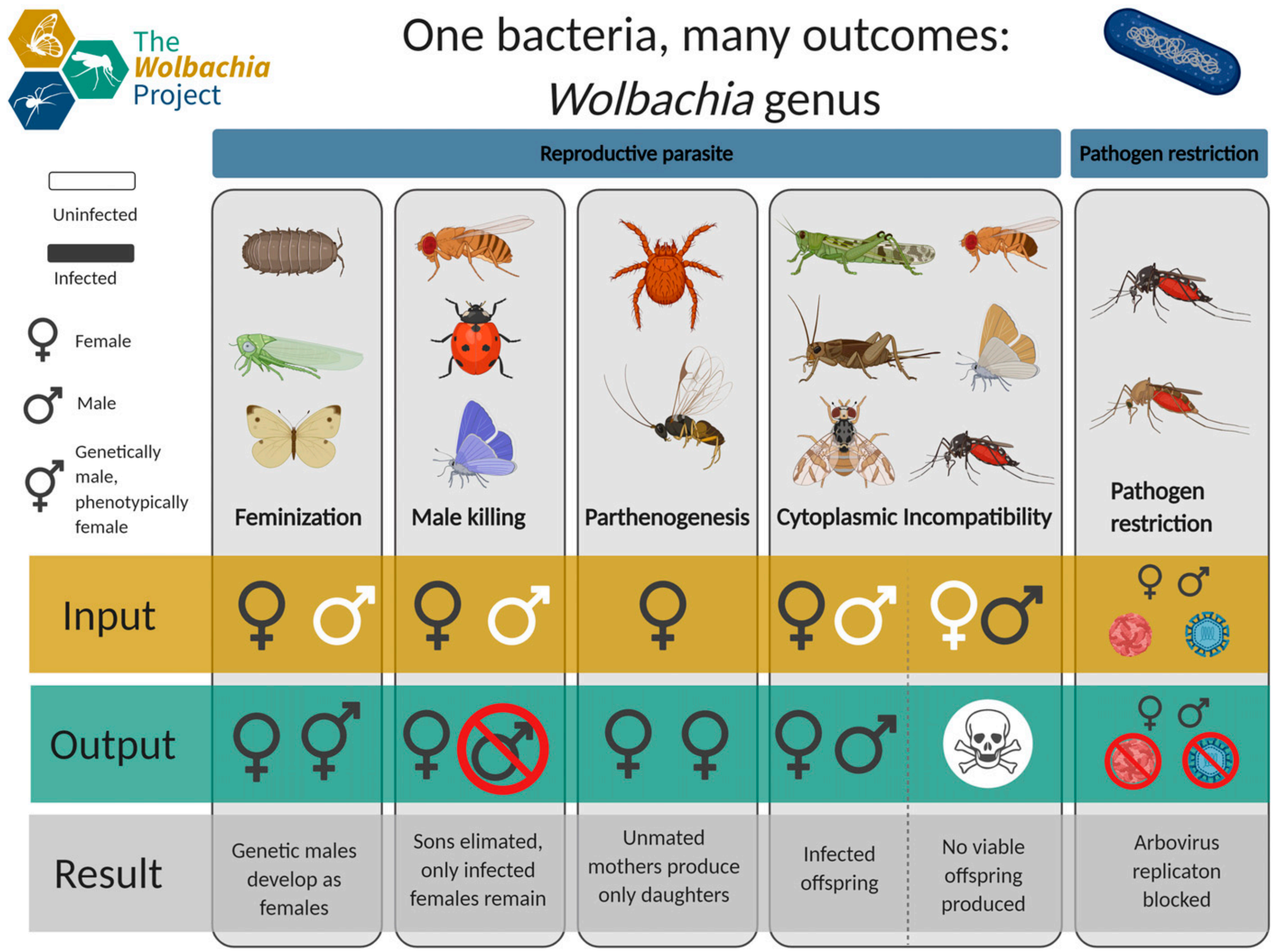

Figure 1 *Wolbachia* cause a fascinating variety of arthropod manipulations that aid their spread or assist vector control. *Wolbachia* are primarily transmitted through the eggs of female arthropods, and resultantly *Wolbachia* enhance their spread by modifying host sex ratios, sex determination, and embryonic viability to increase the relative fitness of infected females (the transmitting sex) via four major reproductive parasitism phenotypes: feminization, male killing, parthenogenesis, and cytoplasmic incompatibility. These phenotypes can affect the evolution of arthropod populations in diverse ways including the evolution of reproductive isolation barriers or change in arthropod mating rituals. In addition to the parasitic impacts of *Wolbachia*, they can block pathogens such as Zika and dengue viruses in some mosquito species. This pathogen restriction phenotype is used globally to curb the spread of arbovirus transmission to humans. These and other features offer compelling reasons for teachers, students, citizen scientists, and researchers to study *Wolbachia* in arthropods. Figure created with BioRender.com.

15 years ago by high school educators and *Wolbachia* colleagues including Seth Bordenstein, Michael Clark, Bob Minckley, Jack Werren, and George Wolfe, and it was deeply enhanced by science educators, among them Christine Brothers, Michele Bahr, and Sarah Bordenstein who engaged in the project at an early time for discovery-based research in the classroom (Bordenstein *et al.* 2010).

Wolbachia as a Research Topic and a Tool for Education

The bacterial genus *Wolbachia* is an ideal candidate for student and citizen exploration and discovery for four principal reasons. First, we live in a microbial world. The human holobiont (ourselves, our microbiome, and virome) is composed of half human cells and half microbial cells, and there are 10^2- to 10^3-fold more genes in the human microbiome than there are in the human genome (Qin *et al.* 2010; Tierney *et al.* 2019). Second, this microbial world extends to the flies that land on your fruit, backyard mosquitoes, and basement spiders, all of which naturally harbor the endosymbiotic bacteria *Wolbachia*. *Wolbachia* occur in approximately half (Hilgenboecker *et al.* 2008; Zug and Hammerstein 2012; Weinert *et al.* 2015) of the million named and seven million estimated arthropod species (Ødegaard 2000; Zhang 2013; Stork *et al.* 2015). Research scientists cannot sample all of these host species nor discover new lineages of *Wolbachia* on their own. Students and citizen scientists, however, increase

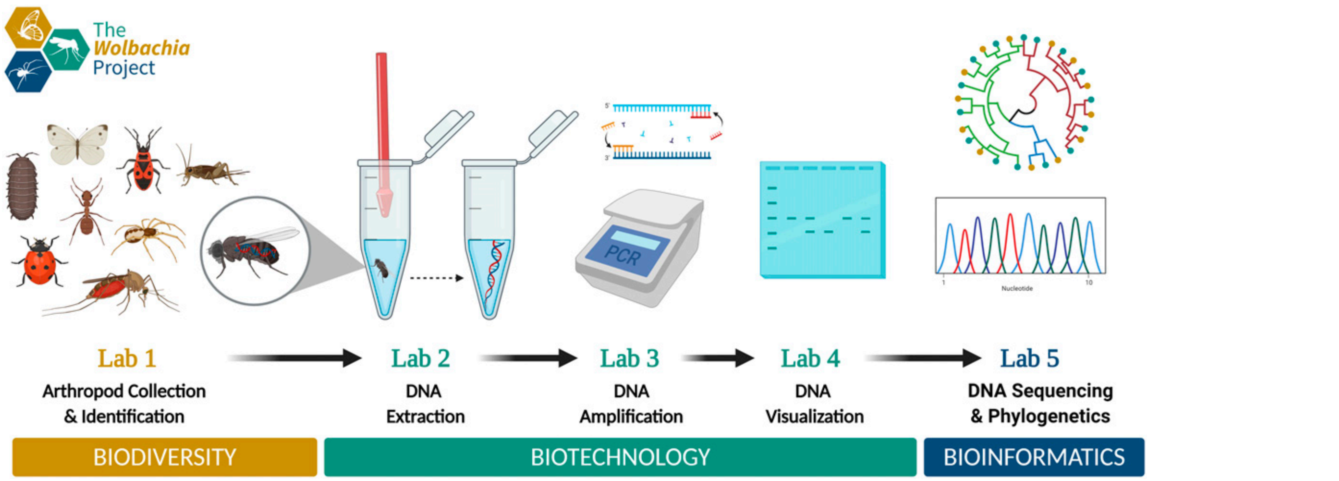

Figure 2 The *Wolbachia* Project enables hands-on scientific exploration in and outside classrooms. The lab series can be broken down into three overarching components: biodiversity, biotechnology, and bioinformatics. Participants begin the project by collecting and identifying local arthropods while learning about *Wolbachia*. Then, they hypothesize if their arthropod is *Wolbachia* positive. Next, participants extract DNA from their arthropod and the provided arthropod controls, amplify the DNA by PCR using the *Wolbachia* Project's provided primers, and visualize their DNA by running an agarose gel. The participants analyze their controls to confidently answer their original hypothesis. Classrooms and citizen scientists also have the option to sequence the bacterial and arthropod DNA, compare their sequence with previously published sequences, and further construct a phylogenetic tree. Figure created with BioRender.com.

the size of the investigator pool and help determine the breadth of arthropods that can be colonized by *Wolbachia* as well as the genetic novelty and diversity of *Wolbachia* within various hosts. Third, *Wolbachia* specialize in infecting the cells of host reproductive tissues where they cunningly hijack reproduction and cause reproductive parasitism to increase the number of infected females, the transmitting sex of *Wolbachia* (Figure 1) (Taylor *et al.* 2018). This single genus of bacteria can selectively kill males, turn genetic males into morphological females, cause female parthenogenesis, and induce embryonic lethality in crosses between infected males and uninfected females (LePage and Bordenstein 2013; Landmann 2019). Fourth, the presence of *Wolbachia* in arthropods has real-world impacts on human and public health. For example, *Wolbachia* block the replication of various RNA viruses including dengue, West Nile, and Zika (Hedges *et al.* 2008; Johnson 2015; Caragata *et al.* 2016; Flores and O'Neill 2018), and they are actively utilized in vector control efforts across the globe to reduce arbovirus transmission (Teixeira *et al.* 2008; Terradas and McGraw 2017; O'Neill 2018).

Taken together, *Wolbachia* have powerful lessons and applications for the biological world, from teaching major concepts in microbiology, genetics, evolution, and symbiosis to advancing human disease control. Moreover, the topical emergence of microbes, the ubiquity but unknown extent of *Wolbachia* in arthropod hosts, the importance of *Wolbachia* for public health, and its safety to use in the classroom make it a unique and ideal biological system of nested, interacting organisms for students, teachers, and citizen scientists to engage in worldwide.

Hands-on Genetics and the Scientific Process

The *Wolbachia* Project presents an opportunity for participants to take a proactive role in their education and self-identity as scientists. Each investigator selects, collects, and identifies arthropods in their own environment, whether it be their backyard, built environment, nearby park, or schoolyard (Figure 2). Participants make observations on the local biodiversity and create hypotheses relating to whether their arthropod is *Wolbachia* positive or negative. The *Wolbachia* Project provides access to techniques that are common in a biological research laboratory but may not be common in a classroom or citizen scientist setting. This hands-on exposure to the scientific process and method continues as investigators amplify *Wolbachia* and arthropod-specific DNA via polymerase chain reaction (PCR), using primers and positive and negative controls supplied by The *Wolbachia* Project. They then visualize their amplified DNA by gel electrophoresis using rigs with blue light that detect DNA in real time, giving investigators insight into how molecular biology techniques produce results and data. Participants learn about the essentiality of controls to decipher contamination and errors in the processes, and they apply necessary troubleshooting skills while self-reporting their confidence of experimental results. Finally, they answer their original hypothesis: if their arthropod harbors *Wolbachia* or not. To continue their exploration, DNA can be submitted for Sanger sequencing of the specific *Wolbachia* 16S ribosomal RNA (rRNA) gene and arthropod-specific cytochrome oxidase I (COI) gene. Participants are responsible for analyzing the quality of their DNA sequence and conducting a bioinformatics analysis that includes a BLAST homology search to compare their sequences

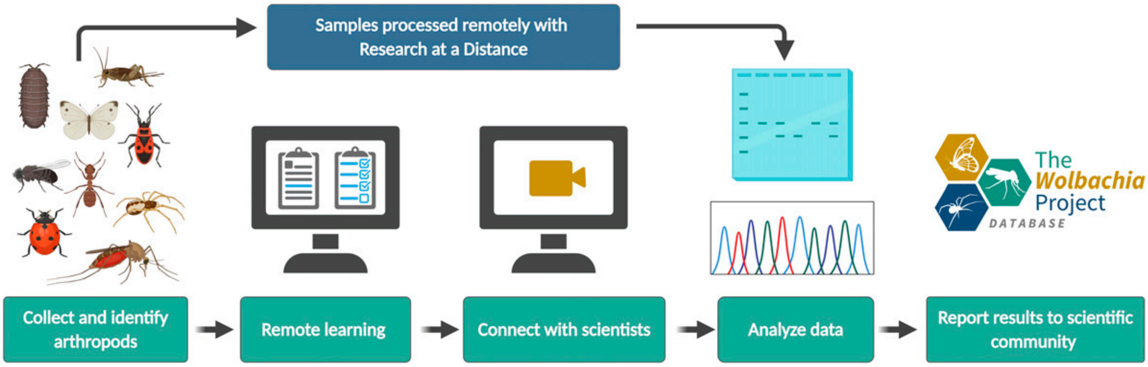

Figure 3 The *Wolbachia* Project proposes five components of virtual learning to enable online learners to engage in science outside of the traditional classroom setting. Participants begin with collection and identification of arthropods in their environment. Subsequently, they engage in "Research at a Distance" in which samples are shipped to and processed by the *Wolbachia* Project team. Students utilize remote learning to learn and virtually practice techniques such as DNA extractions, PCR, and gel electrophoresis, followed by video conference calls with *Wolbachia* scientists. Upon receipt of gel images and DNA sequences from their collected arthropods and *Wolbachia*, students then assess their original hypothesis using the gel images, and they conduct homology and phylogenetic analyses using their sequences. Finally, they share their data with the world by uploading location, specimen, infection status, and sequence data to The *Wolbachia* Project Database. Figure created with BioRender.com.

to those in NCBI's database. The outcome of The *Wolbachia* Project is to enable and share the whole process of scientific exploration, from sample collection to data analysis and critical reflection.

Engagement with the wider global scientific community begins as each investigator asks if their *Wolbachia* sequence, arthropod, or *Wolbachia*–arthropod combination is novel to the published sequence databases and literature. If the *Wolbachia*–arthropod combination is novel, participants compare results to other arthropods that harbor *Wolbachia* and hypothesize what transmission routes could lead to very different hosts harboring the same bacterial strain (*e.g.*, horizontal transfer). They are also able to interrogate the genetic diversity of arthropods and *Wolbachia* lineages. During these explorations, investigators participate in the global scientific process and learn that all experimental data – positive and negative – are useful data. In sum, each contributor to community science uses discovery-based learning, the scientific method, molecular biology techniques, data entry and management, and evolutionary analyses (Figure 2).

Digital Learning for the Pandemic and Postpandemic Epochs

The *Wolbachia* Project is and has always been committed to engaging teachers, students, and citizen scientists, no matter their location or resource availability. Free controls and loaner equipment are commonly provided. In light of the recent (and ongoing) COVID-19 pandemic, the project recently pivoted to provide unique online content and resources that bridge the gap between student research at home and in the classroom (Figure 3). "Research at a Distance (RaaD)" is a new initiative that invites all learners to participate in The *Wolbachia* Project when online learning might prohibit hands-on, research-based activities. The biotechnology component is provided through a partnership between The *Wolbachia* Project and The Vanderbilt Microbiome Initiative (https://vu.edu/microbiome). Participants still collect and identify arthropods and explore the biodiversity in their communities, but their arthropod samples are sent to *Wolbachia* scientists for processing. Meanwhile, they are able to access a digital library to virtually practice and learn about DNA extraction, PCR, gel electrophoresis, Sanger sequencing, and evolutionary informatics using a curated selection of online simulations, videos, and interactions. Students have the opportunity to connect with *Wolbachia* scientists to see an active research laboratory and the techniques used to process their arthropod samples. Data are returned as an image of the agarose gel along with a four-color Sanger sequencing chromatogram of the specific arthropod COI gene and, if applicable, *Wolbachia* 16S rRNA gene for analysis. The workflow then returns to the student's hands in which they must interpret the data and use NCBI's BLAST database to compare findings with other arthropod and *Wolbachia* sequences. Finally, all results are reported to the scientific community by uploading data to The *Wolbachia* Project Database (https://wolbachiaprojectdb.org), a new tool to collect arthropod–*Wolbachia* observations and molecular biology data in one repository.

The database enables participants to practice their data input and management skills while increasing global scientific communication among schools and the research community. Beyond data uploads, students can use the database to develop and test hypotheses. For example, how many ladybugs are *Wolbachia* positive in my area? Is there an arthropod that is more likely to be sampled? Importantly, online learning tools for The *Wolbachia* Project are designed to meet the remote challenges of school and to use critical thinking, data visualization, and genetic analysis during this unprecedented time.

Classroom Collaboration: Within and Beyond

The *Wolbachia* Project reaches thousands per year, and no *Wolbachia* Project classroom is without support. Scientists and educators offer assistance and guidance, and classroom collaborative partnerships can form, such as the international bridge established between a science teacher in Toms River, NJ and a colleague in Israel. High school scientists captured mosquitoes in their area, tested them for the presence of *Wolbachia*, and video conferenced to compare data with students across the world. Students not only practiced their critical thinking and scientific reasoning, but they also participated in a global collaboration. A student from that cohort shared "*It was a great and impactful learning curve in my life. The skills I learned from this research will forever impact my life*."

Next Generation STEM Literacy

It has become increasingly clear that science, scientists, and a scientifically literate public are crucial as we face the impacts of pandemics, global climate change, loss of biodiversity, and increasingly data-centric and technology-based jobs. The importance of science education persists into our adult lives. In 2016, the General Social Survey published by the National Opinion Research Center at the University of Chicago reported 52% of Americans who had not completed high school said they believe science does more good than harm, while 84% of those with bachelor's degrees and 94% of those with graduate degrees expressed this same view. Furthermore, only 30% of Americans said they had a clear understanding of what is meant by a "scientific study," despite scientists being one of the most trusted sources of information (National Science Board 2018). It is vital for STEM literacy and the appreciation and practice of science to expand not only into classrooms with resources, but also into underserved, underrepresented, and rural areas. After participating in The *Wolbachia* Project, a student in Nashville, TN shared "*Science is so much more than just what you see in a textbook and on TV*."

The *Wolbachia* Project warmly welcomes teachers, students, citizen scientists, and mentors of all ranks to connect with *Wolbachia* scientists. We encourage you and your local school district to Discover the Microbes Within! with The *Wolbachia* Project. To get involved or learn more about the project, visit https://vu.edu/wolbachia and our social media venues.

Literature Cited

Bordenstein, S. R., C. Brothers, G. Wolfe, M. Bahr, R. L. Minckley *et al.*, 2010 Using the *Wolbachia* bacterial symbiont to teach inquiry-based science: a high school laboratory series. Am. Biol. Teach. 72: 478–483. https://doi.org/10.1525/abt.2010.72.8.3

Caragata, E. P., H. L. C. Dutra, and L. A. Moreira, 2016 Exploiting intimate relationships: controlling mosquito-transmitted disease with *Wolbachia*. Trends Parasitol. 32: 207–218. https://doi.org/10.1016/j.pt.2015.10.011

Flores, H. A., and S. L. O'Neill, 2018 Controlling vector-borne diseases by releasing modified mosquitoes. Nat. Rev. Microbiol. 16: 508–518. https://doi.org/10.1038/s41579-018-0025-0

Hedges, L. M., J. C. Brownlie, S. L. O'Neill, and K. N. Johnson, 2008 *Wolbachia* and virus protection in insects. Science 322: 702. https://doi.org/10.1126/science.1162418

Hilgenboecker, K., P. Hammerstein, P. Schlattmann, A. Telschow, and J. H. Werren, 2008 How many species are infected with *Wolbachia*? – a statistical analysis of current data. FEMS Microbiol. Lett. 281: 215–220. https://doi.org/10.1111/j.1574-6968.2008.01110.x

Johnson, K. N., 2015 The impact of *Wolbachia* on virus infection in mosquitoes. Viruses 7: 5705–5717. https://doi.org/10.3390/v7112903

Landmann, F., 2019 The *Wolbachia* endosymbionts, pp. 139–153 in *Bacteria and Intracellularity*, edited by P., Cossart, C. R. Roy, and P. Sansonetti, Wiley, Hoboken, NJ; American Society for Microbiology, Washington, DC. https://doi.org/10.1128/microbiolspec.BAI-0018-2019

LePage, D., and S. R. Bordenstein, 2013 *Wolbachia*: can we save lives with a great pandemic? Trends Parasitol. 29: 385–393. https://doi.org/10.1016/j.pt.2013.06.003

National Science Board, 2018 Chapter 7. Science and Technology: Public Attitudes and Understanding in *Science and Engineering Indicators 2018*. NSB-2018–1. National Science Foundation, Alexandria, VA. Available at https://www.nsf.gov/statistics/indicators

NGSS Lead States, 2013 *Next Generation Science Standards: For States, by States*. National Academies Press, Washington, DC, DOI: 10.17226/18290. https://doi.org/10.17226/18290

Ødegaard, F., 2000 How many species of arthropods? Erwin's estimate revised. Biol. J. Linn. Soc. Lond. 71: 583–597. https://doi.org/10.1111/j.1095-8312.2000.tb01279.x

O'Neill, S. L., 2018 The use of *Wolbachia* by the world mosquito program to interrupt transmission of *Aedes aegypti* transmitted viruses, in *Dengue and Zika: Control and Antiviral Treatment Strategies – Advances in Experimental Medicine and Biology*, Vol. 1062, edited by R., Hilgenfeld, and S. Vasudevan. Springer, Singapore. https://doi.org/10.1007/978-981-10-8727-1_24

Qin, J., R. Li, J. Raes, M. Arumugam, K. S. Burgdorf *et al.*, 2010 A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464: 59–65. https://doi.org/10.1038/nature08821

Stork, N. E., J. McBroom, C. Gely, and A. J. Hamilton, 2015 New approaches narrow global species estimates for beetles, insects, and terrestrial arthropods. Proc. Natl. Acad. Sci. USA 112: 7519–7523. https://doi.org/10.1073/pnas.1502408112

Tanenbaum, C., 2016 *STEM 2026: A Vision For Innovation In STEM Education*, Office of Innovation and Improvement, US Department of Education, Washington, DC.

Taylor, M. J., S. R. Bordenstein, and B. Slatko, 2018 Microbe Profile: *Wolbachia*: a sex selector, a viral protector and a target to treat filarial nematodes. Microbiology 164: 1345–1347. https://doi.org/10.1099/mic.0.000724

Teixeira, L., A. Ferreira, and M. Ashburner, 2008 The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. PLoS Biol. 6: e1000002. https://doi.org/10.1371/journal.pbio.1000002

Terradas, G., and E. A. McGraw, 2017 *Wolbachia*-mediated virus blocking in the mosquito vector *Aedes aegypti*. Curr. Opin. Insect Sci. 22: 37–44. https://doi.org/10.1016/j.cois.2017.05.005

Tierney, B. T., Z. Yang, J. M. Luber, M. Beaudin, M. C. Wibowo *et al.*, 2019 The landscape of genetic content in the gut and oral human microbiome. Cell Host Microbe 26: 283–295.e8. https://doi.org/10.1016/j.chom.2019.07.008

Weinert, L. A., E. V. Araujo-Jnr, M. Z. Ahmed, and J. J. Welch, 2015 The incidence of bacterial endosymbionts in terrestrial arthropods. Proc. Biol. Sci. 282: 20150249. https://doi.org/10.1098/rspb.2015.0249

Zhang, Z.-Q., 2013 Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness (Addenda 2013). Zootaxa 3703: 1–82. https://doi.org/10.11646/zootaxa.3703.1.1

Zug, R., and P. Hammerstein, 2012 Still a host of hosts for *Wolbachia:* analysis of recent data suggests that 40% of terrestrial arthropod species are infected. PLoS One 7: e38544. https://doi.org/10.1371/journal.pone.0038544